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Sulfamethazine advances puberty in male chicks by effecting a rapid increase in gonadotropins[☆]

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Abstract

A sulfonamide, sulfamethazine (SMZ) has been shown to have a robust, progonadal effect. The mechanism of action of SMZ, however, is unknown. Our hypothesis is that the compound may act centrally and/or at the level of the pituitary. Four experiments were completed to test that hypothesis. Chicks exposed to a continuous photoperiod and fed a diet containing 0.2% SMZ showed an exponential increase in testes size. When 6 weeks of age (5 weeks on the SMZ diet), experimentals had testes weight nine times heavier than controls. Profiles for thyroid and gonadotropin plasma hormones suggested that T_3 was transiently lower in experimentals solely during the first week on treatment, while thyroxine levels were not different from controls. In contrast, luteinizing hormone (LH) and follicle stimulating hormone (FSH) were significantly elevated at the initial 1-week sampling point and remained elevated throughout the entire experiment. In a follow-up study, LH was found significantly higher than controls by 48 h after initially consuming the compound. When T_3 was added to the SMZ diet at 0.5 ppm, the progonadal effect of SMZ was attenuated. Importantly, chronic intake of T_3 delayed but did not block the stimulatory effect of SMZ for increasing plasma LH. We conclude that since one of the primary effects of SMZ is to increase rapidly plasma gonadotropins, data suggest the compound is acting at the level of the brain or pituitary to stimulate early gonadal development in chicks.

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1. Introduction

Three techniques have been developed in our laboratory to stimulate gonadal growth in male broiler chicks. One comprised the use of parasagittal hypothalamic knife cuts made from the

preoptic area to the mammillary region of the diencephalon in chicks at 2 weeks of age (Mass and Kuenzel, 1983) while the second involved chronic injections of neuropeptide Y (NPY) into the lateral ventricle of the brain of developing chicks (Fraley and Kuenzel, 1993). The third was adapted from a study originally reported to increase testes size in Leghorn chicks (van Tienhoven et al., 1956). Adding sulfamethazine (SMZ) at a concentration of 0.2% to a chick starter diet effected precocious puberty in broilers (Macko Walsh and Kuenzel, 1997). Semen production can be produced as early as 9–10 weeks posthatching,

[☆] Data collected for this study were obtained from experiments conducted in the Department of Animal and Avian Sciences, University of Maryland, College Park, MD 20742, USA.

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comparable to the surgical procedure involving the hypothalamus that induced rapid testes growth (Mass and Kuenzel, 1983). The mechanism by which SMZ stimulates gonadal development is unknown. Early work suggested that SMZ activated thyroid function thereby influencing gonadal development (van Tienhoven et al., 1956). No assays were available for determining thyroid hormone levels in avian species at the time that study was conducted. We therefore designed experiments to determine changes in thyroid hormones after initiating intake of SMZ. Gonadotropins were also determined since it is known that propylthiouracil, a drug that blocks the conversion of thyroxine (T_4) to 3,5,3'-triiodothyronine (T_3) is effective in rats for stimulating testes development without showing significant changes in gonadotropins (Cooke and Meisami, 1991; Kirby et al., 1992; van Haaster et al., 1992). We hypothesized that SMZ would increase plasma gonadotropins since we have found in a preliminary experiment that SMZ effected an increase in plasma (LH) for each of 3 weeks after consuming the compound (Kuenzel et al., 1997). In addition, SMZ significantly increased the number of NPY neurons in an avian hypothalamic area homologous to the arcuate nucleus of mammals (Macko Walsh and Kuenzel, 1997). It has been previously shown that in ovariectomized rats treated with estrogen and progesterone, NPY significantly augmented plasma luteinizing hormone (LH) release (Kalra and Crowley, 1984).

2. Materials and methods

2.1. Experiment 1: Weekly determinations of testes weight and plasma hormones following SMZ intake

Arbor Acre male broiler chicks from 1 day of age ($n=80$) were raised in Petersime batteries. Food and water were available ad libitum. Birds were kept under continuous light as this is the standard industry practice for raising broiler (meat-type) chicks. Temperature was initially set at 35 °C and lowered 2.5 °C each week. Beginning at 1 week of age, half the broilers were fed a standard broiler starter ration while the remainder were fed a standard diet supplemented with 0.2% SMZ (Sigma Aldrich Co.). Twenty-four hours after consuming the experimental diets and at weekly intervals thereafter, heparinized blood samples were taken from the brachial vein [$n=7$ /treatment

(trt)]. Blood samples were centrifuged at $470\times g$, plasma removed and frozen until assayed for luteinizing hormone (LH) and follicle-stimulating hormone (FSH) according to the radioimmunoassay (RIA) procedures validated for chickens (Krishnan et al., 1993, 1994). Intra- and inter-assay variability for gonadotropins were, respectively, LH: 1.1% and 7.8%; FSH: 4.78% and 8.42%. Assay kits for T_3 and T_4 (Diagnostic Products Corp., CA) were used for performing RIAs for thyroid hormones. Intra- and inter-assay variability were, respectively, T_3 : 3.7% and 4.5%; T_4 : 4.1% and 5.2%.

At 2 weeks of age (birds on experimental diets for 1 week) and at weekly intervals until 6 weeks old, chicks on control ($n=7$) and SMZ ($n=7$) diets were sacrificed, testes dissected and weighed.

2.2. Experiment 2: Daily determinations of plasma hormones following intake of SMZ

Broiler male chicks ($n=24$) were raised in Petersime batteries as described for experiment 1. When 1 week of age, birds were distributed to four pens. At 2 weeks of age, birds in two pens ($n=6$ /pen) were given the standard broiler starter ration, the other two pens of birds were fed the standard diet supplemented with 0.2% SMZ. Chicks in two pens (Control and SMZ) were blood sampled from the brachial vein on days 1, 3, 5 and 7 following time of initiating treatment diets. Birds in the other two pens were sampled on days 2, 4, 6 and 8 of dietary treatments. Plasma samples were frozen until analyzed for LH, T_3 and T_4 .

2.3. Experiment 3: Dose–response effect of dietary T_3 plus SMZ on gonadal development

Broiler male chicks ($n=20$) were raised in a Petersime battery as described for experiment 1. Birds were divided into four pens ($n=5$ /pen). Beginning at 1 week of age, birds were fed one of the following four diets: broiler starter diet (CON), SMZ diet (previously described 0.2% SMZ ration), SMZ diet+0.25 ppm T_3 and SMZ diet+0.5 ppm T_3 . After 3 weeks of consuming experimental diets, birds were killed, testes removed and weighed.

2.4. Experiment 4: Effect of dietary T_3 plus SMZ on testes size and plasma LH

Forty male broiler chicks were raised as previously described. When 1 week of age, birds were

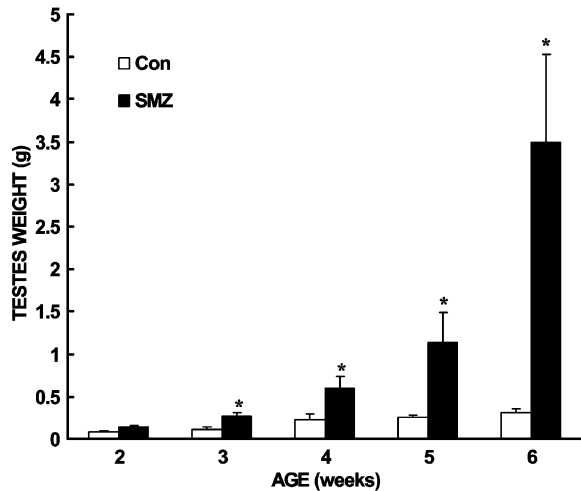


Fig. 1. Testes response to intake of a control or 0.2% SMZ diet. Histograms show mean \pm S.E., $n=7$ /treatment (trt). An * designates a significant difference for that time period between the two trts ($P<0.05$).

distributed to one of four pens ($n=10$ /pen) and fed the following diets: CON diet, CON diet + 0.5 ppm T_3 , SMZ diet and SMZ diet + 0.5 ppm T_3 . Diets were fed for 3 weeks. Blood samples were taken at 1 and 3 weeks on the diets for later analysis of LH. All birds were killed at the end of 3 weeks on dietary treatments. Testes were removed and weighed.

2.5. Statistics

Data were analyzed by the general linear models procedure for analysis of variance. Data were tested for homogeneity of variance. When not homogeneous, data were log-transformed before analysis was performed. Means were separated by the Student–Newman–Keuls procedure using a level of probability of $P<0.05$ (SAS Institute, 1990).

3. Results

3.1. Experiment 1: Weekly determinations of testes weight and plasma hormones following SMZ intake

Results show that testes response to chronic SMZ intake is rapid and exponential (Fig. 1). The equation describing best fit of the data is as follows:

$$Y = 0.14e^{[-5.5 + (1.1)X]}$$

where Y =weight of testes and X =time in weeks consuming the diet. Analysis of variance showed both a significant age and treatment effect. After 2 weeks intake of the experimental diet (3 weeks of age), testes weight of treatment birds was significantly heavier than controls. By 6 weeks of age, SMZ chicks had testes weights nine times the size of controls (Fig. 1). Mean body weight was the same (mean = 119 g) for the two groups of chicks ($n=35$ /group) selected at 1 week of age to begin consuming the experimental diets (SMZ vs. Control diets). At the end of the study, body masses did not differ significantly between the two groups (SMZ birds: 1622 ± 76.01 g; controls: 1631 ± 65.49 g; mean \pm S.E., $P>0.05$).

When plasma LH and FSH were analyzed by ANOVA, both hormones showed significant age and treatment effects. Plasma LH (Fig. 2a) and FSH (Fig. 2b) levels were significantly higher in SMZ-fed chicks compared to controls beginning at 1 week following consumption of treatment diets. Plasma gonadotropins remained elevated in birds consuming SMZ throughout the length of the study.

Plasma T_3 was significantly lower in SMZ-fed birds after 24 h consuming experimental diets (Fig. 2c). Thereafter no differences were observed between the two treatment groups until weeks 5 and 6 where SMZ birds showed significantly higher T_3 values. No significant age or treatment effects were obtained in T_4 values or T_3/T_4 ratios (data not shown).

3.2. Experiment 2 Daily determinations of plasma hormones following intake of SMZ

Due to the rapid rise in gonadotropins (Fig. 2a,b) and the significantly lower plasma level of T_3 in chicks fed an SMZ diet for 24 h (Fig. 2c), the second study focused upon the first week on dietary treatments and included blood samples taken daily. Chicks consuming diets containing SMZ showed significantly higher plasma LH within 48 h of initiating treatments (Fig. 3). The significant rise in plasma LH over the first 4 days was linear and averaged 1.73 ng/ml increase per day (Fig. 3). Thereafter, mean LH values appeared to plateau in birds fed the SMZ diet. In contrast, controls showed relatively steady plasma LH levels approximately 6 ng/ml.

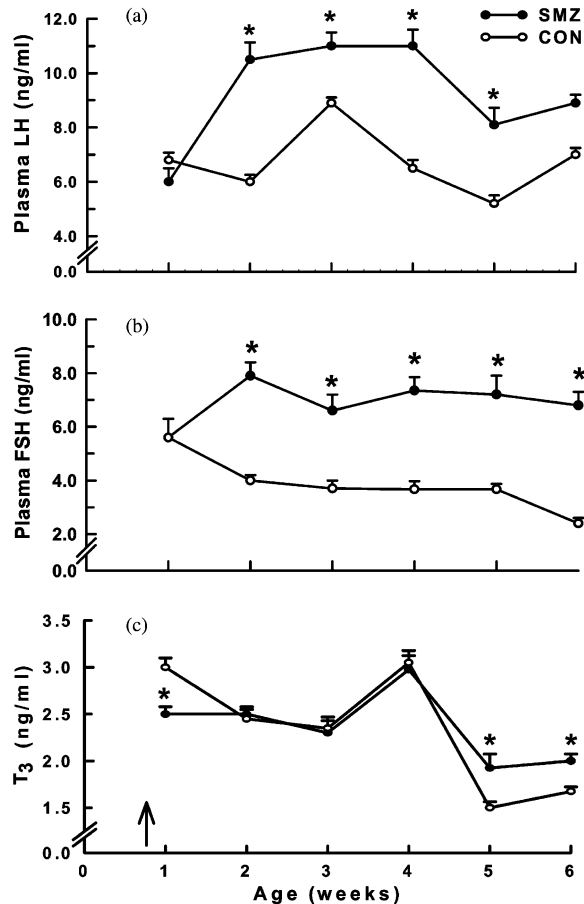


Fig. 2. A,B and C. Weekly mean values of plasma luteinizing hormone (LH), follicle stimulating hormone (FSH) and triiodothyronine (T_3) following intake of a control or 0.2% SMZ diet. Vertical lines show \pm S.E. An * designates a significant difference for that time period between the two treatments ($P < 0.05$). The vertical arrow indicates time when experimental diets were begun. First blood sample was taken 1 day following initiation of dietary treatments.

A second finding in the study was a significant overall treatment effect for plasma T_3 levels. Specifically, chicks fed SMZ had significantly lower T_3 levels across the 8-day sampling period compared to those fed the control diet (Fig. 4). Experimental birds also displayed significantly lower T_3 levels on day 5. Data suggest that transient, low levels of T_3 affected by intake of SMZ may facilitate rapid testes development.

3.3. Experiments 3 and 4: Effect of dietary T_3 plus SMZ on gonadal development and plasma LH

To test the hypothesis that transient, low levels of plasma T_3 may be required for SMZ to effect

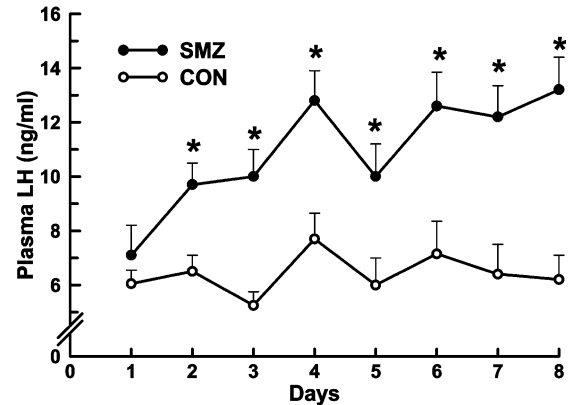


Fig. 3. Daily plasma LH levels following intake of a 0.2% SMZ diet. Vertical lines show \pm S.E., $n=6$ /treatment (trt). An * designates a significant difference for that time period between the two trts ($P < 0.05$).

rapid testes growth, an experiment was designed to add T_3 to an SMZ diet at levels of 0.25–0.5 ppm. Such dietary additions of T_3 to poultry rations have resulted in a 25–50% increase in plasma T_3 (May, 1980). Results of the dose–response experiment for dietary T_3 are shown in Fig. 5. Note that testes weight was not found significantly lower in birds consuming an SMZ diet over a 3-week period with 0.25 ppm T_3 added to it. In contrast, dietary addition of 0.5 ppm T_3 to the experimental diet resulted in a significantly smaller testes weight compared to chicks consuming solely the SMZ diet (Fig. 5). To confirm the result, a 2×2 factorial experiment was completed

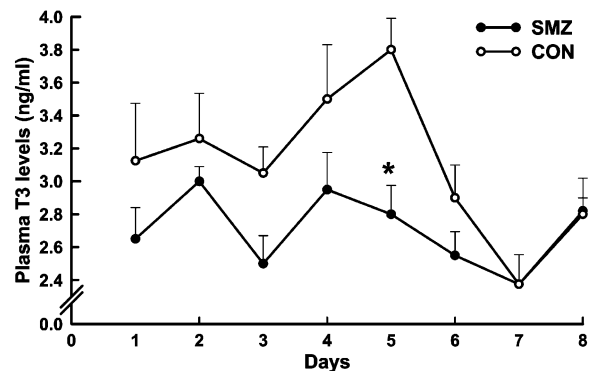


Fig. 4. Daily plasma T_3 levels following intake of a 0.2% SMZ diet. Vertical lines show \pm S.E., $n=6$ /treatment (trt). Analysis of variance showed an overall significant trt effect on T_3 levels ($P < 0.05$). An * designates a significant difference for that time period between the two trts ($P < 0.05$).

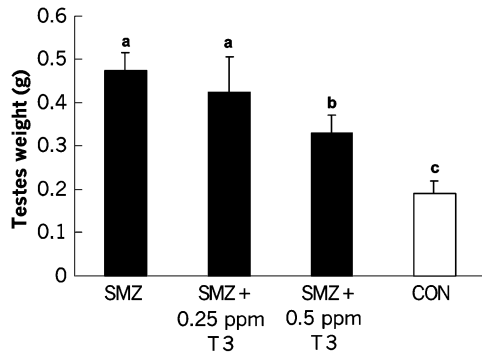


Fig. 5. Dose-response effect of T_3 added to a 0.2% SMZ diet on testes development. Histograms show mean \pm S.E., $n = 5$ /treatment. Treatments with unlike letters are significantly different ($P < 0.05$).

involving two treatment diets (CON and SMZ diets) with and without dietary addition of 0.5 ppm T_3 . Analysis of variance showed a significant effect for SMZ to increase testes weight. In contrast, adding 0.5 ppm T_3 significantly attenuated the progonadal effects of SMZ ($P < 0.05$, Fig. 6). Plasma LH results (Table 1) likewise show that T_3 added to the SMZ diet initially suppressed the expected rise in LH at the end of week 1. Interestingly, when plasma LH levels were determined at the end of the study (week 3, Table 1), there were no significant differences in plasma LH between the SMZ vs. the SMZ + T_3 groups. Results suggest that the addition of 0.5 ppm T_3 to the diet does not completely block testes development, rather it appears to delay gonadal growth expected from the SMZ diet. The initial delay in testes growth may be the result of the significant reduction in plasma gonadotropins during the first week of consuming the SMZ diet containing T_3 . By week 3, however, plasma LH was as elevated in birds consuming the SMZ + T_3 diet as chicks consuming the SMZ diet alone.

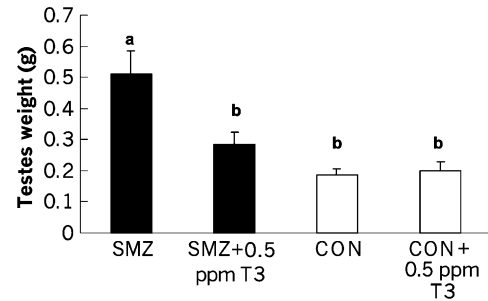


Fig. 6. A 2×2 factorial study showing the effects of SMZ and/or T_3 added to a standard broiler chick ration. Histograms show mean \pm S.E., $n = 10$ /treatment. Treatments with unlike letters are significantly different ($P < 0.05$).

4. Discussion

Adding SMZ to the diet of male broiler chicks (*Gallus gallus*) beginning at 1 week of age resulted in an exponential increase in size of the gonads, confirming past studies conducted in this type of bird (Macko Walsh and Kuenzel, 1997). In attempting to determine the possible mode of action of SMZ for stimulating testes development, gonadotropins were measured. Of interest was that LH was shown to be significantly elevated within 48 h of consuming the diet containing SMZ (Fig. 3) and continued to rise over the first 4 days of blood collection with an average rate of increase of 1.73 ng/day. The response is quite comparable to reported values of LH in wild avian species when they have been transferred from a short-day to a long-day photoperiod. The LH assay has been a technique used for many years to assess the photosensitivity of avian species (Follett and Davies, 1975). Specifically, it has been well documented that many avian species occupying the Northern temperate zone, both wild and domestic, respond to a long-day photoperiod with rapid development of the gonads (Farner and Wilson, 1957; Etches, 1996). A rapid rise of LH has been

Table 1
Plasma luteinizing hormone (LH, ng/ml) levels in broiler chicks following consumption of four different diets

Dietary treatments	One week on treatment	Three weeks on treatment
Control diet (CON)	$4.4 \pm 0.47^{1,a}$	3.9 ± 0.67^a
CON + 0.5 ppm T_3	2.2 ± 0.68^a	2.5 ± 0.89^a
Sulfamethazine (SMZ)	9.1 ± 1.25^b	7.5 ± 0.67^b
SMZ + 0.5 ppm T_3	3.5 ± 0.86^a	6.2 ± 1.45^b

¹Data show means \pm S.E.; $n = 5$ /treatment. Values with unlike superscripts for each time period differ significantly ($P < 0.05$).

associated with photosensitivity of the proposed photoneuroendocrine axis responsible for effecting gonadal development in birds (Follett and Davies, 1975). Two species known to be highly sensitive to changes in photoperiod, White-crowned Sparrows, *Zonotrichia leucophrys gambelii*, Japanese Quail, *Coturnix coturnix japonica* show a rapid, significant rise in LH within 24 h of transferring birds from a short- to a long-day photoperiod. The average increase in LH over the first 2 days after transfer was 0.85 and 1.23 ng/day for White-crowned Sparrows and Japanese Quail, respectively (Follett and Davies, 1975). Data show (Fig. 3) that the LH response of male broilers fed SMZ was quite comparable to the rapid rate seen in migratory birds exposed to long-day photostimulation as well as other avian species shifted from a short- to a long-day (Sharp, 1993). Therefore, it is plausible that SMZ may operate via the classical photoneuroendocrine system and hence involve photoreceptors located in the eyes, pineal and/or brain (Foster and Follett, 1985; Kuenzel, 1993; Menaker et al., 1970; Vigh-Teichmann et al., 1980; Wada et al., 2000; Wilson, 1991). Further research will be required to test the hypothesis.

Follicle stimulating hormone (FSH), similar to LH, showed a significant increase following initial intake of SMZ (Fig. 2). Due to the rapid rate of testes growth (Fig. 1), and the consistent, significant elevation of FSH throughout the 6-week study suggest that monitoring FSH plasma levels in the future is as important as LH for assessing gonadotropin response from SMZ intake.

It has been reported that SMZ affects thyroid function (van Tienhoven et al., 1956) and that one of its effects in mammals is to inhibit iodination reactions catalyzed by thyroid peroxidase (Doerge and Decker, 1994). Since thyroid hormones have not been determined in birds that have consumed SMZ, plasma concentrations were first determined in experiments 1 and 2. Thereafter, T_3 was added to both control and SMZ diets. Results showed a significant but transient reduction in T_3 during chronic intake of the sulfonamide compared to controls (Fig. 4). More importantly, adding T_3 to the SMZ diet attenuated testes size (Figs. 5 and 6) and delayed the expected rise in plasma LH (Table 1). Data suggest that short-term, low levels of plasma T_3 may be required for maximum rate of gonadal growth following SMZ intake. It has been known for some time that thyroid hormones significantly influence gonadal development

(Goldsmith and Nicholls, 1984; Thapliyal, 1969; Thapliyal and Gupta, 1984; Verheyen et al., 1986; Wieselthier and van Tienhoven, 1972; Wilson and Reinert, 1993). Of relevance to data shown in Fig. 2c and Figs. 4–6 are the data of Verheyen et al. (1986) showing a thyroid-gonadal antagonism related to T_3 . In future studies, it would be important to determine the expression of iodothyronine deiodinase in brain and testes samples to understand better the mechanism of SMZ in stimulating early gonadal development.

In summary, SMZ stimulates an exponential growth of testes in young, male broiler chicks. When fed at 0.2% in a starter ration, the compound causes a rapid rise in plasma gonadotropins and a transient suppression of T_3 . The rapid rise in LH, within 48 h of consuming the SMZ diet, at a time in the life cycle of the chick when testes tissue is barely visible, suggests that SMZ is operating at the level of the brain or pituitary gland. Further studies will be necessary to determine the anatomical locus or loci and specific cellular actions initially caused by this compound that results in the marked, progonadal effect.

Acknowledgments

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